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Synthesis and structural confirmation of selaginpulvilin X†

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We report the first total synthesis of the natural product selaginpulvilin X, a selaginellaceae polyphenol class of compounds. Our synthetic strategy employs cross-coupling reactions and an organolithium addition to construct the carbon framework. Subsequently, the functional group modifications and de-protection yield the natural product. Spectral analysis confirms the proposed structure by comparing natural and synthetic samples.

Selaginellaceae polyphenols¹ are a structurally diverse class of compounds isolated from plants of the *Selaginella* genus, which includes selaginellins,² selaginpulvilins^{3,4} and selaginbenzophenones, amongst others.^{5–8} These ancient species, considered living fossils, have been used in traditional folklore medicines worldwide to treat ailments such as jaundice, gonorrhoea, acute hepatitis, asthma, dysmenorrhoea, and traumatic injuries.^{9,10} Recent studies have demonstrated that extracts from these plants exhibit a variety of biological effects, including anticancer, anti-inflammatory, antimicrobial, antioxidant, and antiviral activities in both *in vitro* and *in vivo* settings.^{11–13} Additionally, the biological activity of several selaginella polyphenols, including selaginpulvilins^{3,14} and selaginbenzophenones,^{6,7} as well as their unnatural derivatives,^{15–17} has been confirmed by our research and that of others.

The structural peculiarity and biological activity of the isolated compounds attracted the attention of organic and medicinal chemists, including our research group. We have reported the synthesis of selaginpulvilin A and D,¹⁸ as well as selaginbenzophenones A (1) and B (3).¹⁹ Additionally, we discovered that

the compound previously described as selaginbenzophenone B was incorrectly identified and is actually selaginbenzophenone A. Our findings prompted us to investigate the structural features of other representatives of this family and we carried out a synthesis of selaginbenzophenone C (2) and confirmed the proposed structure of the natural product (Fig. 1a).²⁰ Recently, Zhu and co-workers reported the isolation of a new *Selaginellaceae* polyphenol, selaginpulvilin X (5).²¹

The naturally isolated compound features a biphenyl system, a benzophenone unit, and a diaryl acetylene in a 1,2,3 substitution (Fig. 1b). Alkynyl polyphenol compounds, includ-

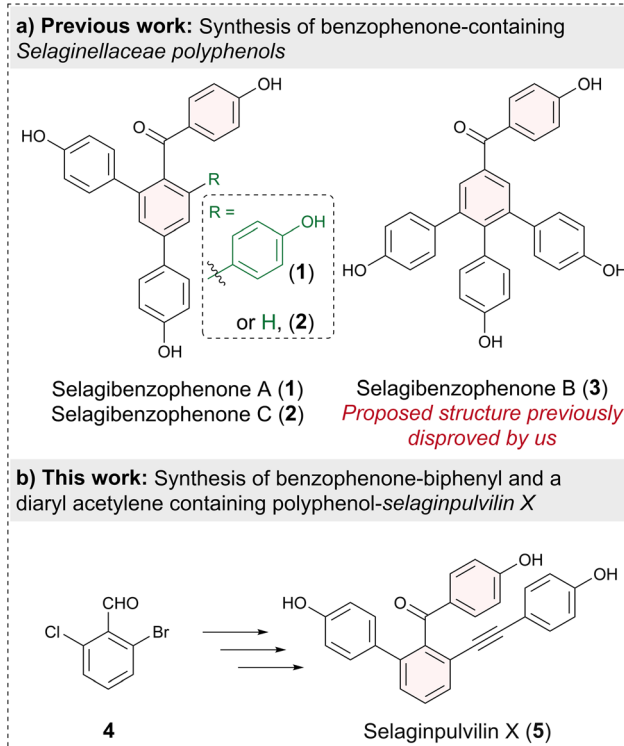


Fig. 1 Current work in the context of previous research.

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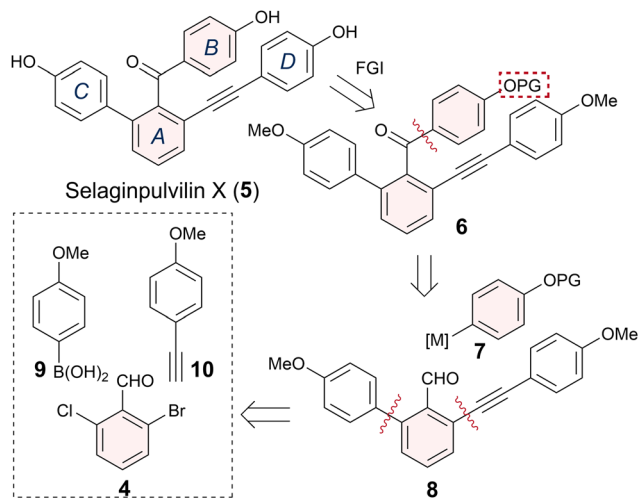


Fig. 2 Retrosynthetic analysis.

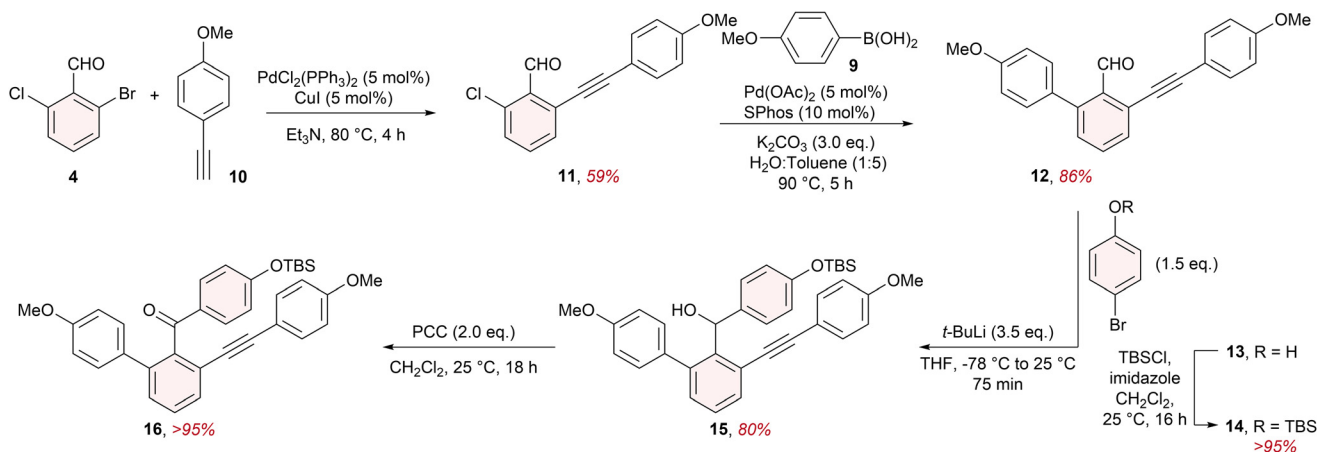
ing, but not limited to selaginellins are renowned for their diverse biological activities.^{22–28} Given these findings and our previous work on the structural discrepancies of some *Selaginellaceae* polyphenols, we aim to carry out the first total synthesis of selaginpulvilin X (5) and confirm its proposed structure by comparing the spectral characteristics of the isolated and synthetic compounds.

Retrosynthetically, the synthesis can be achieved through the global deprotection of compound 6. Special attention must be paid to the use of an easily cleavable protecting group for the phenol at aromatic ring B to avoid the demethylation issues recently observed by our group¹⁹ and also reported by Wang during the synthesis of selaginpulvilin X analogues.²⁹ An organometallic addition to an aldehyde 8, and the functionalization of 2-bromo-6-chlorobenzaldehyde 4, whose substitution pattern matches that of the natural compound completes the retrosynthetic analysis (Fig. 2).

In the first straightforward step, the alkynyl moiety is introduced *via* Sonogashira cross-coupling reaction with commer-

cially available 4-methoxyphenylacetylene (10) (Scheme 1). This reaction is performed in freshly distilled triethylamine, using 5 mol% of $\text{PdCl}_2(\text{PPh}_3)_2$ and CuI as the catalytic system. After 4 hours at 80 °C, the reaction yielded 59% of the desired alkyne 11, with the alkynyl moiety regioselectively introduced at the bromine position of the starting aldehyde 4. In the next step, chloride of compound 11 was subjected to a Suzuki cross-coupling. In our initial attempt, the substrate was treated with 4-methoxyphenylboronic acid (9) in the presence of the XPhosPdG2 catalyst. To our delight, the reaction provided the desired coupling product 12 in a reasonable 53% yield. Furthermore, we have attempted to increase the reaction yield by changing the palladium source to $\text{Pd}(\text{OAc})_2$, while keeping the XPhos ligand resulted in a formation of the desired product, which was isolated in 51% overall yield along with some unknown inseparable impurity. Nevertheless, changing the ligand to SPhos significantly increased the yield of the desired product 12 to 86%. However, it is noteworthy to mention, employing SPhos ligand from various vendors had a negative impact on the reaction and led to a drop of the yield and formation of side products, which contaminated the desired aldehyde 12. We even attempted the recrystallization of the ligand from a new source, however without success. In the next step, we introduced the aromatic ring B *via* the addition of organolithium species, *in situ* generated from the corresponding arylbromide. To avoid using a methoxy-protected analogue, we opted for the TBS-protected 4-bromophenol 14, which was synthesized with an 95% yield by TBS protection of 4-bromophenol (13).

This decision was based on our experience, as well as that of others, who encountered difficulties in removing the methoxy group from the *para* position to the keto group.^{19,29} Treatment of the bromide 14 with *t*-BuLi (3.5 eq.) resulted in the *in situ* generation of the aryllithium species, which underwent a smooth addition to aldehyde 12, and provided secondary alcohol 15 in 80% yield. Further oxidation of the alcohol to ketone using PCC resulted in the formation of orthogonally protected selaginpulvilin X 16 in >95% yield.



Scheme 1 Synthesis of the carbon framework of the natural product.



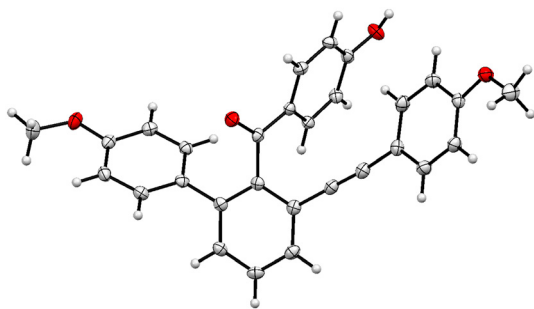


Fig. 3 View on molecule **17** with the displacement ellipsoids at 50% probability level. CCDC number: 2376335.

We considered two approaches for the overall deprotection: first, cleaving the TBS group before demethylation, or alternatively, removing the methoxy groups before deprotecting the TBS group. We first explored the former approach and subjected ketone **16** to 1.2 equivalents of TBAF (Scheme 2). This successfully yielded monophenol **17** in 87% yield. A crystal suitable for X-ray crystallography was obtained through the slow evaporation of a d_4 -MeOD NMR sample of **17**, and the X-ray structure is depicted in Fig. 3. Subsequently, we attempted the deprotection of the methoxy groups. Compound **17** was treated with BBr_3 (2.2 eq.) and the analysis of the reaction mixture revealed the presence of the desired product **5**, however, in only trace amounts. The majority of the material underwent unspecified decomposition. The absence of the alkyne signals in the ^{13}C NMR spectra suggested that the alkyne was too reactive to withstand the reaction conditions. We then attempted to deprotect the methoxy groups from the

fully protected compound **16**. However, the outcome was nearly identical to that with phenol **17**, with only trace amounts of the desired product detected.

The unsuccessful attempts to deprotect the methoxy groups prompted us to explore alternative deprotection methods. Based on a report of Fang *et al.*, we subjected phenol **16** to LiCl in DMF at $160\text{ }^\circ\text{C}$ under microwave irradiation, but no conversion was observed.³⁰ Finally, treating the fully protected compound **16** with sodium ethanethiolate, freshly prepared from ethanethiol and metallic sodium, resulted in the formation of the natural product **5** in a moderate 43% yield.

Finally, with the synthesized natural product in hand, we proceeded to compare its spectral characteristics with those of the reported compound isolated from the plant. Analysis of both the ^1H and ^{13}C NMR spectra revealed a strong correlation between the two (see ESI[†]). Both spectra exhibited the characteristic doublets for the symmetrical protons of the aromatic rings B, C, and D, as well as signals corresponding to the central aromatic core A. The chemical shifts were consistent in both ^1H and ^{13}C NMR spectra.

Conclusions

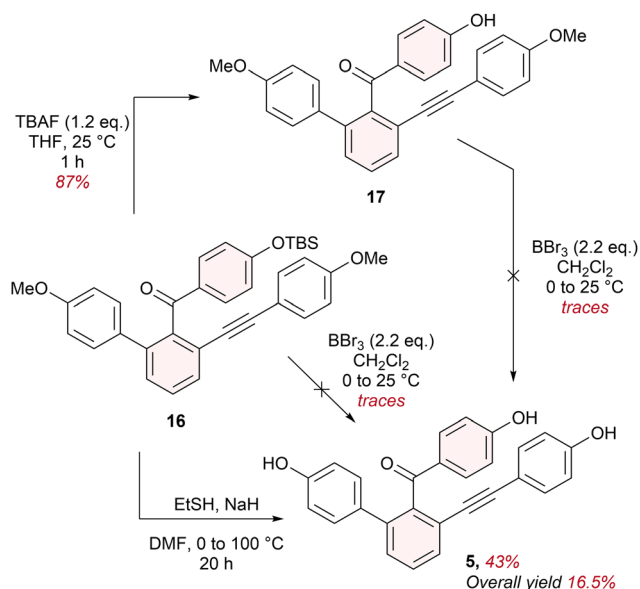
In conclusion, we achieved the first total synthesis of the natural product selaginpulvilin X (**5**). The synthesis was accomplished in five linear steps in an overall 17% yield, including Sonogashira and Suzuki cross-couplings, the addition of aryl lithium species to an aldehyde, oxidation to the ketone, and global deprotection. Our work confirms the proposed structure of the natural product through a comparison of the NMR spectral characteristics of the synthetic and natural compound, as well as by X-ray crystallography of an advanced intermediate of the synthesis. The developed synthetic strategy also provides access to various methylated derivatives, complementing previously published protocols. This offers a robust platform for potential biological evaluations and structure–activity relationship studies.

Author contributions

TH: investigation, methodology, writing – original draft and writing – review & editing; SN: investigation, methodology, writing – original draft and writing – review & editing; IC: investigation; LR: conceptualization, data curation, funding acquisition, project administration, resources, supervision, visualization, writing – original draft, and writing – review & editing.

Data availability

The data underlying this study are available in the published article and its online ESI.[†]



Scheme 2 Finalizing the synthesis of selaginpulvilin X.



Conflicts of interest

There are no conflicts to declare.

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